Supplementary Figure Legends

Supplementary Figure 1. Comparison of the quantification performance of SRM, regular PRM and IS-PRM analyses of a dilution series of 93 SIL peptides spiked in a plasma sample. All 558 fragment ions selected / monitored in the various methods were evaluated. The numbers of transitions that could be used for reliable quantification at different dilution points were indicated.

Supplementary Figure 2. Number of SIL peptides systematically detected in triplicated IS-PRM analyses of a dilution series of 93 SIL peptides spiked in a plasma sample. The numbers of peptides satisfying the spectral matching acceptance criteria used in the present study at each dilution point were indicated.

Supplementary Figure 3. Comparison of cycle times in regular PRM ("PRM-method A" and "PRM-method B") and IS-PRM analyses of 606 pairs of SIL and endogenous peptides in a plasma sample on a *Q-Exactive HF* instrument. The cycle times observed over the entire analyses were plotted together with the corresponding numbers of peptides monitored in the regular PRM analyses. The average cycle time values between 15 and 52 min, corresponding to the elution range of 578 of the pairs of peptides were also displayed, corresponding to 8, 2.8, and 2 s for "PRM-method A", "PRM-method B", and IS-PRM, respectively.

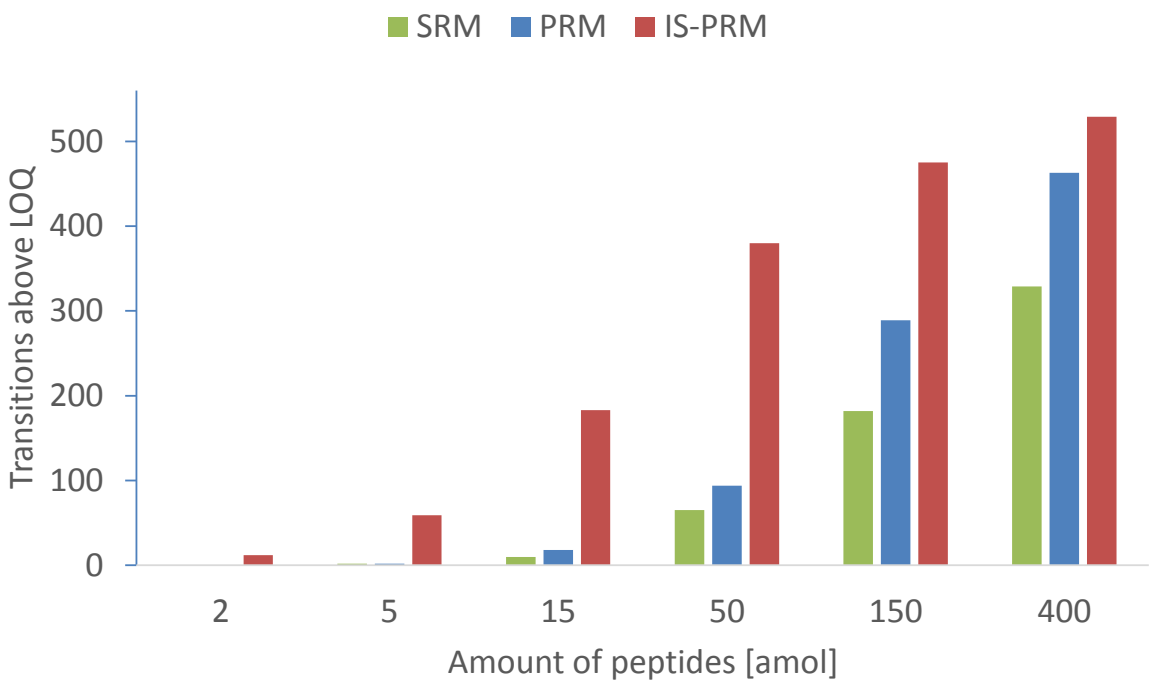
Supplementary Figure 4. Detection of targeted endogenous peptides in plasma, urine, and HeLa cell samples by triplicated IS-PRM analyses of 606 pairs of SIL and endogenous peptides. A) The Venn diagram illustrates the overlap between the sets of peptides detected in the different samples (in at least one of the triplicated analyses). B) The heatmap reflects the peak areas of the 606 endogenous peptides in the different samples (see **Supplementary data 5**). A peak area value of zero was considered for the peptides not detected (reported in white) or not systematically detected (reported in grey) in a sample. For the peptides systematically detected in a sample, an average AUC was calculated (based on the sum of the AUC of their six fragment ions in triplicated analyses) and reported based on a blue color scale. This scale was defined based on the percentiles of the entire dataset, *i.e.*, including the (non-zero) average AUC of all the peptides measured in each sample. C) Extracted fragment ion traces for six selected endogenous peptides exhibiting large differences in their abundance between the three samples.

Supplementary Figure 5. Detection of targeted endogenous peptides in a chimeric sample, prepared by mixing in equal proportion plasma, urine, and HeLa cell samples, by triplicated IS-PRM analyses of 606 pairs of SIL and endogenous peptides. A) Two heatmaps were prepared to reflect the peak areas

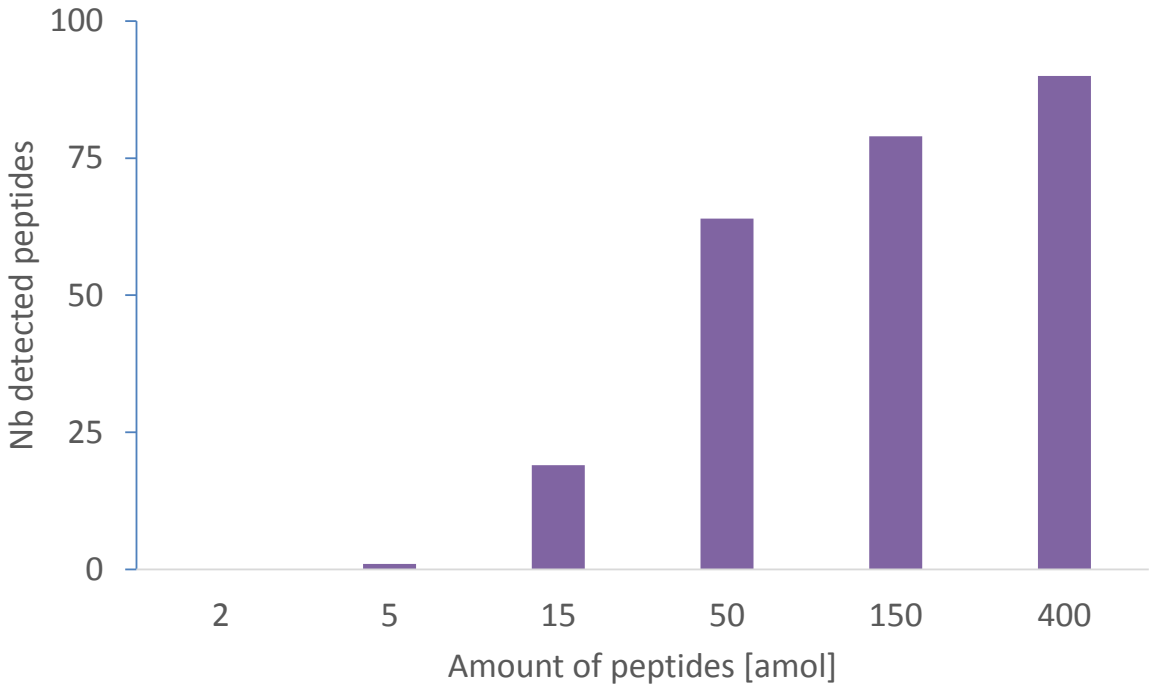
of the endogenous peptides in the chimeric sample based either on the results of the actual analyses of the sample ("experimental") or of the individual sample analyses ("predicted"). The peak area values of the peptides reported in the "experimental" heatmap were obtained by applying to the chimeric sample analyses the same data processing procedure as that described in **Supplementary Figure 4** for individual sample analyses (see **Supplementary data 5**). In the "predicted" heatmap, the peak area values were estimated by averaging the peak areas calculated at the level of individual sample analyses (reported in **Supplementary Figure 4**). The peptides detected or systematically detected in none of the samples, thus being associated with a peak area value of zero, were distinguished by a white or grey color code, respectively. For both heatmaps, the peak area values were reported using the same blue color scale as that used in **Supplementary Figure 4** (based on the results of the individual sample analyses). B) Extracted fragment ion traces for three selected endogenous peptides systematically detected in only one of three individual samples and in the chimeric sample.

Supplementary Figures

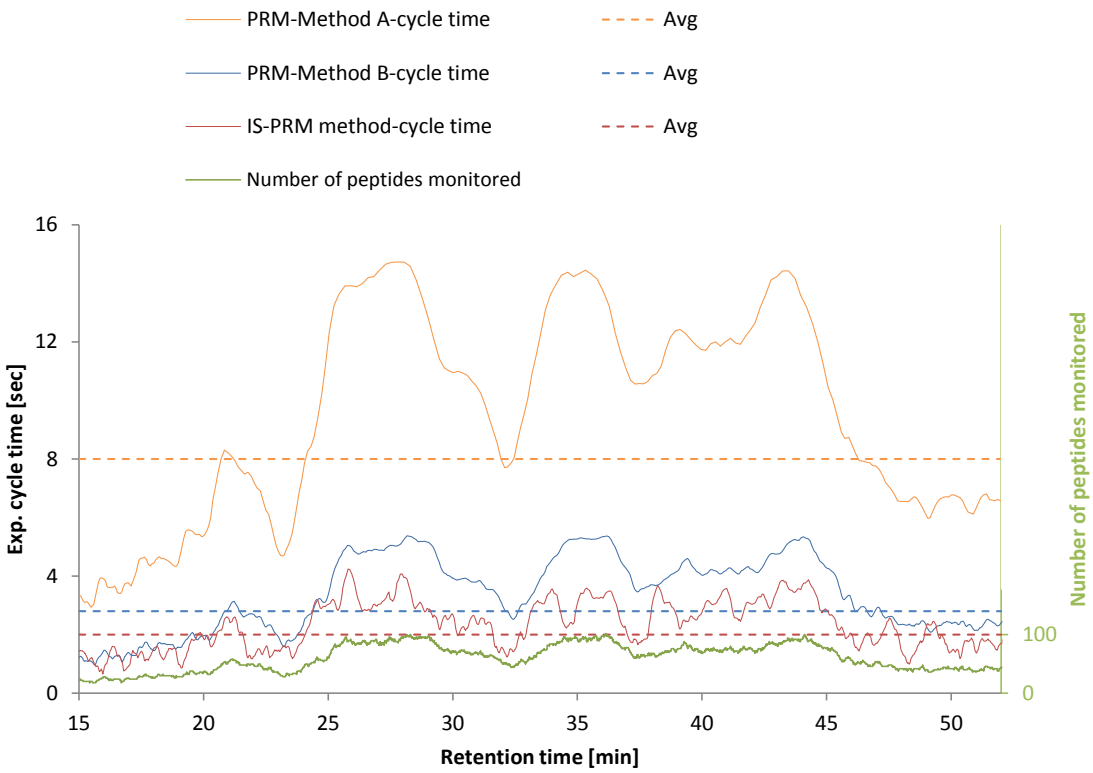
Supplementary Figure 1



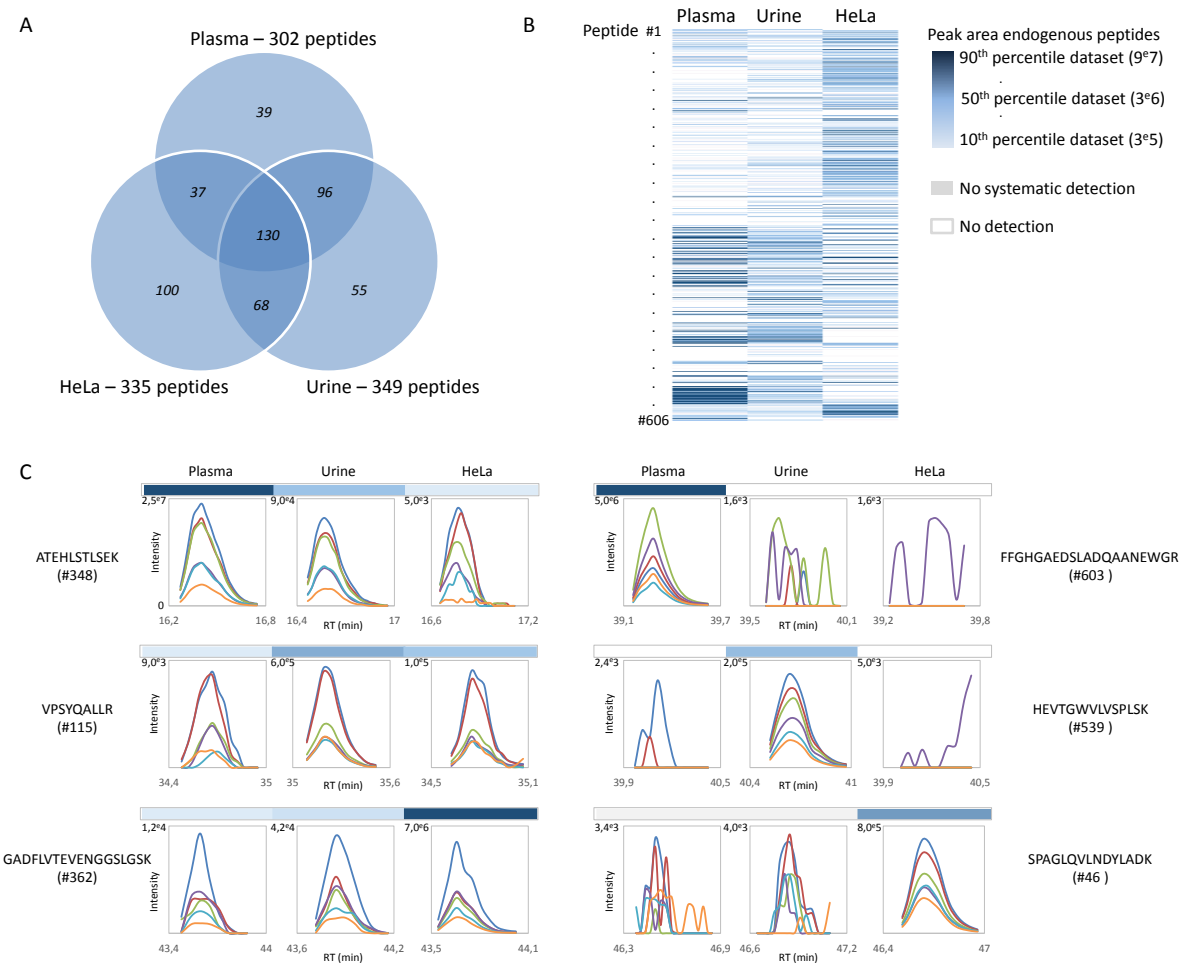
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5